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Title: A Novel Nonchemical Method for Quarantine Treatment of Fruits: California Red Scale on Citrus

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Citation: Journal of Economic Entomology (2004) 97:(6) 1861-1867

Number: 7481

A Novel Nonchemical Method for Quarantine Treatment of Fruits: California Red Scale on Citrus

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ABSTRACT A process for removing or killing California red scale, *Aonidiella aurantii* (Maskell), from citrus fruit as a postharvest treatment was evaluated. The process subjects the fruit to vacuum, steam, and vacuum that physically removes red scale from the fruit and kills those scales that are not removed from the fruit. Different numbers of cycles and steam temperatures were compared for efficacy in removing scale from lemons or killing those that remained. Multiple (two to three) cycles removed up to 96% of first molt scales on the fruit, but they were much less effective in removing other stages, especially those that had advanced beyond the second instar. However, it was extremely effective in killing the scales remaining on the fruit. Although this process does not eliminate cosmetic damage caused by scale presence, it might be used in combination with high-pressure washers currently used in packing houses to allow importers and exporters to meet the most stringent quarantine requirements. Because of its killing power, this technique should be tried on other insects and commodities to see whether it can be substituted for certain uses of methyl bromide.

KEY WORDS California red scale, *Aonidiella aurantii*, citrus, nonchemical control, vacuum-steam-vacuum

THE LIKELY LOSS OF methyl bromide in the near future requires that alternative methods of quarantine treatment for agricultural commodities be developed for application to the tens of millions of dollars of products imported and exported each year. In addition, alternatives are needed for the many commodities that do not tolerate methyl bromide treatment, presenting unresolved quarantine problems. Several alternative technologies, such as new fumigants, irradiation, controlled atmospheres (Morgan and Gaunce 1975), and hot or cold temperatures have been developed for elimination of insect pests and other unwanted organisms on agricultural commodities, but most require a lengthy treatment period, and resistance has been reported for some of the fumigants (Zettler et al. 1989). The current study represents an initial trial of the vacuum-steam-vacuum (VSV) surface intervention process, a technology originally developed to eliminate harmful microorganisms on the surface of food stuffs (Goldberg et al. 2001, Kozempel et al. 2002), for quarantine treatment against insects.

Bacterial contamination of fruits and vegetables is usually confined to the surface of whole, undamaged solid food (Gill and Penney 1977). The VSV process was designed to rapidly treat the surface of solid foods with condensing steam to kill bacteria without damaging the product, for example fruits and vegetables (Kozempel et al. 2002), chicken (Goldberg et al. 2001; Kozempel et al. 2003a, b), and hot dogs (Kozempel et al. 2000). This is accomplished by applying vacuum to remove the resistance films of air and moisture, followed by a 0.1-s application of saturated steam, followed by vacuum to effect evaporative cooling to avoid any thermal damage from the steam. The VSV process has been successfully applied to fruits and vegetables without thermal damage in our pilot plant unit (Kozempel et al. 2002). Because the process is effective in killing bacteria hidden in the surface pores, we hypothesize that the VSV process should be able to penetrate and kill surface infestation of solid foods by insects.

For this initial trial, we chose the California red scale, *Aonidiella aurantii* (Maskell), as a test insect, because it is an important postharvest pest (Walker et al. 1999) and thus receives substantial field insecticide applications (Morse and Klonsky 1994), which can diminish the effectiveness of biological control agents on *A. aurantii* and other citrus pests as well (Haney et al. 1992). Postharvest removal of this scale is generally accomplished by high-pressure washing, although this

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process can sometimes damage some fruit (Walker et al. 1996, 1999). Because scales are a quarantine problem on many different commodities (Witherell 1984, Follett and Gabbard 1999), as well as a cosmetic pest on fruits (Walker et al. 1996), we wished to explore the potential of VSV both as a removal process and killing agent for California red scale. In this article, we provide data from experiments designed to evaluate the effectiveness of the VSV process in removing and killing California red scale.

Materials and Methods

Source of Infested Fruit. All tests used Lisbon lemons, *Citrus limon* L., infested with California red scale. The first experiment used scale-infested lemons supplied by the University of California, Riverside. All fruit were selected by size and color from citrus blocks that had been pesticide free for at least 3 mo. Lemons were cold stored at 8°C (45°F) for at least 2 d to minimize cell damage to the rind during washing. Lemons were washed and rinsed in large tubs by using an elongated bottlebrush and standard 1.6-cm (5/8-in.) garden spray nozzle. Once lemons were dry and at ambient room temperature, each lemon had the calyx removed and was half dipped (covering blossom and stem end of fruit) in melted household paraffin colored with Sudan III fatty acid dye. The wax seals half of the fruit, which prolongs the quality of the fruit for the host insect (up to 8 wk). The dye aids the visual inspection and the inoculation process. Once the fruit were waxed, each fruit was hand brushed and inspected with a 10× head lens to remove all field scale and parasitoids. At this point, the fruit were inoculated with insectary reared California red scale by using a modified Tashiro method (Tashiro 1966). Our modification, using 2 h for inoculation instead of 24 h, prevented excessive scale buildup. Once scales on the infested lemons reached the desired stage(s), they were shipped by overnight courier to the USDA-ARS Beneficial Insects Introduction Research Unit at Newark, DE, where they were prepared for experimentation. Seven such shipments with scales ranging from first instars to gravid females were provided.

In the second experiment, we used F_1 progeny from lemons used as controls in the first experiment. Lemons of undetermined variety were purchased in local (Newark, DE) supermarkets and infested by allowing crawlers from the control lemons to climb up cardboard tubes and settle on the lemons placed thereon.

VSV Surface Intervention Processor Mechanical Design. The surface intervention processor was designed to process chicken carcasses, specifically broilers, but it is applicable to other solid food products. The performance requirements are to accept product samples individually and enclose them in a chamber within a rotor. Then, in sequence, evacuate the chamber to vacuum; close the vacuum valves and subject the product to saturated steam; close the steam valves and draw vacuum on the chamber to evaporatively cool the product; and finally eject the sample into a clean environment. To accommodate samples of lemons infested with red scale, a cylindrical wire basket was installed. The basket was 152 mm in diameter and 152 mm in depth. The basket was enclosed with screening that had 12-mm openings. The spherical chamber (product valve) is 254 mm in diameter.

To create a vacuum or admit steam into the closed chamber, two opposed 200-mm holes were bored through the ball valve stator (housing) at right angles to both the axis of rotation of the ball and to the centerline of the open chamber (product entry and exit ports). Two platter valves are close coupled to these 200-mm ports. Each consists of a flat disk with two holes or ports rotating against an inlet header, which holds polyetheretherketone seals. When the disk is aligned with the ports in the inlet header, gas flows into or out of the treatment chamber. Multiple holes reduce the disk angular movement necessary for valve action and increase the cross-sectional area for gas flow.

Each disk is programmed and controlled independently and moved by its own servomotor. The servos are by Allen-Bradley Co., Inc., Mayfield Valley, OH, and capable of high acceleration and deceleration. The servos for the disks are model 1326AB-C4B-11, 5.6 kW capable of 1,600 rpm maximum. The servos are direct coupled mechanically to the disks. Operation of the servos, by Graphics Motion Language (GML) software version 3.8.2, Allen-Bradley Co., Inc., Mayfield Valley, OH, controlled the vacuum and steam times. Data acquisition was by Laboratory Technologies Corp., Lab Tech Notebook version 8.04, Wilmington, MA. Sensors were by Omega Engineering, Inc., Stamford, CT. Type E thermocouples were used for temperature, and Omega PX176 series sensors were used for vacuum and for steam pressure.

Vacuum was supplied by a liquid ring vacuum pump, Stokes Vacuum model HER, Philadelphia, PA. The steam generator was a 115-liter tank consisting of horizontal submerged coils with no separator and 17.8-kW heaters. It was made in-house and charged with tap water, which was boiled for 30 min for deaeration. The normal saturated steam temperatures used were 138 and 143°C. The vacuum receiver was adjusted to 7 kPa and its condenser coil cooled to 4°C.

VSV Surface Intervention Processor Operation. Each sample was manually inserted into the treatment chamber of the VSV surface intervention processor. The computer-controlled ball valve was rotated, with a servo, 90° to seal the chamber from the outside atmosphere. The platter valves in the main chamber rotated to expose the sample to vacuum, steam, and then vacuum again. With multiple cycles, the sequence of vacuum then steam was repeated multiple times. After treatment, the ball valve rotated 90° to expose the sample to atmosphere. The sample was aseptically removed manually after treatment.

Experimental Procedure. *Experiment 1.* Seven shipments of 50 lemons, each received 7–10 d apart and bearing predominantly different scale stages (first instar-first molt, second instar-second molt, third instar females-prepupal males, and gravid females), were tested. Lemons from each shipment were subjected to

five treatments: one VSV cycle at 138°C, two cycles at 138°C, three cycles at 138°C, one cycle at 143°C, and a control. The initial vacuum time was 0.1 s; steam times were 0.1 s; intermediate vacuum time was 0.1 s; the final vacuum time was 0.5 s. Saturated steam was used. Before treatment, each lemon was numbered, and an area ≈3.5 cm in diameter on the equatorial area of the unwaxed portion of each fruit was encircled with a permanent black marker to define a statistically representative number of scales per sample and to reduce handling of the fruit. In most cases, 50–160 scales would be circumscribed in this manner. Pretreatment counts of scales within the circle were made with a dissecting microscope. Lemons were randomly divided into control and treatment groups, but a modified approach was taken in that the process was repeated if scale densities in the different groups were grossly disparate. On the next day, the lemons were transported to the Eastern Regional Research Center at Wyndmoor, PA, for the actual testing. On each of the seven trips, the control lemons were transported to Wyndmoor, but they did not go through the VSV apparatus. After treatment, fruit (treated and controls) were placed individually in 473-ml unwaxed paper cartons with clear plastic lids and returned to the Newark laboratory where they were stored at 25°C, 60–70% RH, and a photoperiod of 14:10 (L:D) h for evaluation. Post-treatment counts were made the next day to determine the percentage of scales that had been removed from each lemon. Fruit was checked for general quality and presence of mold two to three times a week, and moldy fruit were discarded. Final counts to evaluate the mortality of scales remaining on the fruit were made 25–34 d later. Although most scales receiving VSV treatment were clearly dead, we allowed enough time for scales to progress to the next stage to make final counts, so that the likelihood of a false scoring of dead would be minimal. The descriptions presented in Forster et al. (1995) were used to distinguish the different stages of scales examined.

Experiment 2. The scales used in this experiment were F_1 progeny from the scales on control lemons in experiment 1. Different exposure periods were used to obtain three groups of lemons infested with different stages as follows: first instar-first molt, second instar-second molt, and third instar females-prepupal males. In most cases, 50–200 scales were circumscribed for pretreatment counts. The lemons were unwaxed and subjected to two treatments: one cycle at 138°C, two cycles at 138°C, or controls on a single test date. In other respects, the procedures were identical to those used in experiment 1.

Statistical Analyses. Three variables of interest were selected for analysis: 1) the proportion of scales removed by the VSV process = (pretreatment count − post-treatment count)/pretreatment count; 2) proportion of scales killed by VSV process (i.e., those not alive or emerged males) = (post-treatment count − final live count)/post-treatment count; and 3) total scale destruction = (pretreatment count − final live count)/(pretreatment count). In experiment 1, the data were calculated for each lemon and subjected to two-way analysis of variance (ANOVA) by using the dominant stage (test number) and treatment as grouping factors. Variables with significant dominant stage-treatment interactions were reanalyzed with seven one-way ANOVAs, one for each dominant stage (test). In experiment 2, one of the treatment-stage combinations (first instar-first molt and one cycle at 138°C) was not run, so a two-way ANOVA was not appropriate, and a one-way ANOVA with the remaining combinations as grouping factors was used instead. Parametric tests on data from both experiments were done with STATISTICA version 6.0 (STATISTICA Reference Guide, StatSoft, Inc. 2001).

Results

Experiment 1. Two-way ANOVAs for all scale removal and mortality were highly significant, but treatment effects ($F > 90.13$; df = 4, 254–291; $P < 0.0001$) were much higher than those for scale stage ($F > 2.98$; df = 6, 254–291; $P < 0.01$) and the stage-treatment interaction ($F > 3.25$; df = 6, 254–291; $P < 0.001$). Because interactions were significant for all criteria, individual one-way ANOVAs were done for each dominant stage.

Removal of Scales. The results of tests comparing one VSV cycle at 138°C, two cycles at 138°C, three cycles at 138°C, one cycle at 143°C, and a control were similar across all stages (Table 1). With the exception of test 7, which consisted primarily of third instars (mostly female), the proportion of total scales remaining after treatment differed significantly between the untreated controls and all of those receiving VSV treatment. On that particular date, there were mechanical problems, and only half the usual number of lemons could be tested. (It has to be kept in mind that this VSV unit was a research prototype and, as such, subject to frequent changes and development. It is not unexpected to have frequent mechanical problems in a pilot plant setting. The pilot plant is the venue to discover the problems. It is impossible to give a good estimate of the frequency of maintenance and repair for a commercial unit. However, preliminary data from the development of the commercial VSV unit for hot dogs shows few mechanical problems). Thus, there was a significant difference between the control lemons and those subjected to one VSV cycle at 138°C, but none of the other contrasts differed significantly. Proportions of scales removed tended to be higher on lemons subjected to two or three VSV cycles compared with those receiving only one cycle, but significant differences were noted in only two tests, 1 (first molts) and 4 (first molts-second instars). Proportions of scales removed tended to be higher on lemons infested with early stages (first instars-second instars) of *A. aurantii* than in those with later stages, but this trend was not consistent across all treatments (Table 1). Although scale removal exceeded 90% in some of the multiple cycle treatments, overall rates of removal for single cycle VSV treatments were lower, so scale removal for all VSV treatments combined averaged

Table 1. Percentages (mean ± SEM) of California red scales removed from lemons by vacuum-steam-vacuum process, experiment 1

Treatment		Test no., dominant stage, *n*, and percentage removed (mean ± SEM)						
		3 First instar	1 First molt	4 First molt-second instar	2 Second molt	5 Third instar prepupal ♂♂	7 Third instar females	6 Gravid females
138°C, 3 cycles	*n*	497	1,342	935	670	1,522	377	2,119
	%	97 ± 2a	97 ± 1a	91 ± 3a	83 ± 4a	69 ± 6a	38 ± 11a	73 ± 6a
138°C, 2 cycles	*n*	484	1,335	993	373	1,766	380	1,745
	%	96 ± 2a	91 ± 3a	88 ± 4a	86 ± 4a	64 ± 7ab	20 ± 4ab	63 ± 3a
138°C, 1 cycle	*n*	576	1,518	1,173	620	1,685	385	1,098
	%	86 ± 5a	68 ± 5b	61 ± 7b	58 ± 8a	62 ± 8ab	26 ± 10ab	56 ± 6a
143°C, 1 cycle	*n*	496	1,360	959	568	1,429	389	1,379
	%	82 ± 7a	82 ± 6ab	59 ± 9b	54 ± 7a	39 ± 10b	27 ± 0ab	67 ± 3a
Control	*n*	509	1,367	951	574	1,463	385	1,424
	%	23 ± 4b	11 ± 2c	6 ± 4c	22 ± 4b	3 ± 1c	0 ± 0b	8 ± 2b

Means within a column followed by same letter are not significantly different ($P = 0.05$; Tukey's honestly significant difference test [Spjotvoll and Stoline 1973]).

only 66.4% across all seven tests. Some scales on the control lemons also were removed, apparently by handling, but in most cases, the proportion removed was rather low (Table 1).

Scale Mortality. In most cases, it was easy to distinguish scales killed by the VSV process. The body contents usually exploded, leaving a poorly defined yellowish smear beneath the scale cover. Some scales in both treatments and controls were desiccated, so it is unclear to what extent the VSV process contributed to desiccation. Scales on lemons subjected to VSV treatments did not progress in their development. For example, male prepupae and pupae did not transform into adults. Likewise, gravid females did not yield viable crawlers.

The VSV process was highly effective in killing the scales that were not removed from the fruit, and no scales survived the process (Table 2). Substantial proportions (>50%) of scales on the control lemons in some of the tests died. Apparently, this was caused by density-dependent effects (crowding or fruit deterioration), because it occurred in trials where total scale numbers exceeded 1000 (Table 2). However, in every test but test 7, mortality in these controls differed significantly from those in each of the treatments. In this test, four of the five lemons subjected to one cycle at 143°C became moldy before final counts could be made leaving only one lemon in that treatment group. The apparently substantial difference in scale mortality between the control lemons (21%) and the remaining (100%) lemon subjected to one cycle at 143°C (Table 4) could not be discerned with ANOVA. Therefore, we used a χ^2 test to determine whether the proportion of dead scales differed significantly for this contrast; the proportion of dead scales was clearly higher in lemons subjected to one cycle at 143°C ($n = 48$) than in the controls ($n = 385$; $\chi^2 = 136.78$, df = 1, $P < 0.001$).

Because of the 100% mortality of scales on lemons receiving VSV treatments, the tabulations of the proportions of total scales destroyed (Table 3) were much the same as those for the scales not removed from fruit (Table 1). Elevated scale destruction in some of the controls (Table 3) no doubt reflected the density dependence alluded to above. We should point out that the means presented in Tables 1 and 2 do not add to those presented in Table 3, because different denominators are used in calculating the means. In ad-

Table 2. Percentages (mean ± SEM) of dead California red scales remaining on lemons after treatment, experiment 1

Treatment		Test no., dominant stage, *n*, and percentage removed (mean ± SEM)						
		3 First instar	1 First molt	4 First molt-second instar	2 Second molt	5 Third instar prepupal ♂♂	7 Third instar females	6 Gravid females
138°C, 3 cycles	*n*	10	48	122	80	548	226	648
	%	100 ± 0a	100 ± 0a	100 ± 0a	100 ± 0a	100 ± 0a	100 ± 0a	100 ± 0a
138°C, 2 cycles	*n*	48	165	83	45	603	303	599
	%	100 ± 0a	100 ± 0a	100 ± 0a	100 ± 0a	100 ± 0a	100 ± 0a	100 ± 0a
138°C, 1 cycle	*n*	106	537	615	294	798	279	472
	%	100 ± 0a	100 ± 0a	100 ± 0a	100 ± 0a	100 ± 0a	100 ± 0a	100 ± 0a
143°C, 1 cycle	*n*	101	230	469	271	797	48	458
	%	100 ± 0a	100 ± 0a	100 ± 0a	100 ± 0a	100 ± 0a	100 ± 0a*	100 ± 0a
Control	*n*	274	1,225	895	439	1,418	385	2,406
	%	25 ± 20b	76 ± 4b	37 ± 11b	26 ± 7b	90 ± 4b	21 ± 20b*	73 ± 10b

Means within a column followed by same letter are not significantly different ($P = 0.05$; Tukey's honestly significant difference test [Spjotvoll and Stoline 1973]).

* χ^2 test used to test for significant difference in mortality between control and 143°C, one cycle in test 7.

Table 3. Percentages (mean ± SEM) of California red scales destroyed by vacuum-steam-vacuum process, experiment 1

Treatment		Test no., dominant stage, n, and percentage removed (mean ± SEM)						
		3 First instar	1 First molt	4 First molt-second instar	2 Second molt	5 Third instar prepupal ♂♂	7 Third instar females	6 Gravid females
138°C, 3 cycles	n	456	1,342	935	651	1,522	377	2,119
	%	100 ± 0a	100 ± 0a	100 ± 0a	100 ± 0a	100 ± 0a	100 ± 0a	100 ± 0a
138°C, 2 cycles	n	484	1,335	993	373	1,766	380	1,745
	%	100 ± 0a	100 ± 0a	100 ± 0a	100 ± 0a	100 ± 0a	100 ± 0a	100 ± 0a
138°C, 1 cycle	n	576	1,518	1,173	599	1,685	385	1,098
	%	100 ± 0a	100 ± 0a	100 ± 0a	100 ± 0a	100 ± 0a	100 ± 0a	100 ± 0a
143°C, 1 cycle	n	496	1,360	959	521	1,429	66	1,379
	%	100 ± 0a	100 ± 0a	100 ± 0a	100 ± 0a	100 ± 0a	100 ± 0ab	100 ± 0a
Control	n	396	1,367	951	574	1,463	385	1,424
	%	47 ± 14b	79 ± 4b	41 ± 11c	43 ± 7b	90 ± 4b	21 ± 20b	49 ± 22b

Means within a column followed by same letter are not significantly different ($P = 0.05$; Tukey's honestly significant difference test [Spjotvoll and Stoline 1973]).

dition, means for scales removed (Table 1) included data from lemons that later molded, whereas those for other means (Tables 2 and 3) did not.

Experiment 2. One-way ANOVAs (used instead of two-way ANOVA because of unbalanced design) for all scale removal and mortality data were highly significant ($F > 4.42$; df = 7, 86–89; $P < 0.001$).

Removal of Scales. Despite the high F value (4.42), the differences in proportions of scales removed in VSV treatments and controls were not very great, and only the proportion of first instars-first molts subjected to one VSV cycle at 138°C (46.5%, the highest mean observed) differed significantly from the controls (Table 4). In general, overall levels of scales removed were lower than in experiment 1 (Table 1), even when only the analogous treatments were considered.

Scale Mortality. All scales remaining on lemons subjected to VSV treatments were killed, but mortality was low on the control lemons, and all contrasts between treatments and controls were statistically significant (Table 4). As in experiment 1, the proportions of total scales destroyed merely reflected those remaining on lemons that were killed. For the most part, scale stage did not seem to be very important, except in the case of total scales destroyed, where somewhat higher (but nonsignificant) proportions of third instars on controls were removed and killed than their first- and second-stage counterparts, resulting in an intermediate level of total scale destruction that differed significantly from both the early stage controls and all the VSV treatments. This could have resulted from density-dependent action, because the scale counts on these lemons were much higher than on the controls for other stages (Table 4).

Effect of Treatments on Lemons. The only obvious effect on lemons receiving the VSV treatment was that of dimple reversal; that is, the dimples on the rind became convex instead of concave. Because scale crawlers generally settle in the dimples and begin the sedentary phase of their development there, we believe that this created a gap at the scale cover–fruit interface that facilitated removal of the scales by vacuum and increased mortality by the penetration of steam and additive effects of vacuum at the close of the VSV cycle.

Of the 326 lemons used in experiment 1, 11 (3.3%) became moldy before making final counts. A three-way log linear analysis (Sokal and Rohlf 1981) was applied to the data testing for stage × treatment, stage × fate, treatment × fate, and stage × treatment × fate interactions in mold incidence. Only the stage (test no.) × fate interaction was statistically significant ($G^2 = 23.502$, df = 1, $P < 0.001$), so the results did not indicate that VSV either prevented or

Table 4. Numbers tested, percentages (mean ± SEM) of scale removal, post-treatment mortality, and total scale destruction by treatment (138°C steam or control) and dominant stage, experiment 2

Item determined	Treatment	First instar-molt		Second instar-molt		Third instar-prepupae	
		n	%	n	%	n	%
Removal of scale	2 cycles			816	30.4 ± 7.8ab	3,747	40.4 ± 22.1ab
	1 cycle	644	46.5 ± 9.1b	1,219	35.4 ± 6.5ab	3,851	36.8 ± 11.4ab
	Control	215	3.8 ± 3.4c	446	6.1 ± 3.1a	2,399	16.5 ± 5.3a
Post-treatment Mortality	2 cycles			411	100.0 ± 0.0b	400	100.0 ± 0.0b
	1 cycle	365	100.0 ± 0.0b	726	100.0 ± 0.0b	1,345	100.0 ± 0.0b
	Control	209	0.0 ± 0.0a	420	1.0 ± 1.0a	1,744	34.5 ± 8.3a
Total scale Destruction	2 cycles			610	100.0 ± 0.0c	771	100.0 ± 0.0c
	1 cycle	644	100.0 ± 0.0c	1,219	100.0 ± 0.0c	2,579	100.0 ± 0.0c
	Control	215	3.8 ± 3.5a	446	7.1 ± 3.1a	2,399	49.9 ± 8.0b

Means within a matrix followed by same letter are not significantly different ($P = 0.05$; Tukey's honestly significant difference test [Spjotvoll and Stoline 1973]).

predisposed lemons to mold. However, in experiment 2, none of the 49 lemons in the controls became moldy, whereas six (12%) of the 51 lemons receiving VSV became moldy. Given the small cell sizes, Fisher's exact test (Sokal and Rohlf 1981) was applied to the 2 by 2 contingency table and indicated that the proportion of observations in the different categories defining the table differed significantly from that expected by random occurrence ($P = 0.027$), suggesting that the VSV predisposed the lemons to mold.

Discussion

Although substantial proportions of scales were removed by several VSV treatments, especially those comprising two or three cycles, only a few trials resulted in >90% removal, and the overall results fell far short of those obtained by high-pressure washing, which generally exceed 95% (Walker et al. 1999). Thus, VSV does not seem to represent a promising approach for scale removal for citrus varieties used at this time. However, other, newer varieties of citrus may experience damage due to high-pressure water washing.

We used lemons in this study largely because of convenience (well adapted culture and efficient rearing system), but the VSV process is applicable to many different fruits and vegetables, not just lemons. For instance, the process successfully reduced bacteria levels 2.5–4 logs on kiwis, avocados, cantaloupes, papaya, grapefruit, cucumbers, mangoes, carrots, beets, and peaches (Kozempel et al. 2002). Unfortunately, it was not successful on all produce. It destroyed bananas, peppers, and broccoli. As a killing agent, however, VSV holds considerable potential; all of the trials in this initial study with California red scale resulted in 100% scale mortality. With such high mortality, there would seem to be latitude to use even lower temperatures or vacuum dwells than those used in our study. Although the effects of VSV treatments on the lemons did not seem to be too drastic, it remains to be seen how they would affect shelf life and conform to consumer requirements. One of our two experiments suggested that VSV might increase the incidence of moldy fruit, and sap exudate resulting from scale removal has been implicated as contributing to moldy fruit by other investigators (Giliomee and Swanepoel 1979, Bedford 1990, Du Toit Pelser 1993). However, it should be pointed out that the fruit were not refrigerated or washed, procedures that presumably would greatly reduce or eliminate the incidence of mold to begin with. In addition, the VSV process leaves residual condensed moisture on the fruit. Removing this moisture before shipping or storage should further reduce or eliminate the incidence of moldy fruit. Given the effectiveness of VSV as a killing agent for California red scale, it might possibly be used in combination with high pressure washing to meet both low thresholds for cosmetic damage and the levels of scale mortality needed to meet stringent export or import requirements. Exploratory trials of this technology with other insects and commodities would seem to be indicated.

In a commercial process, lemons would not be treated individually. In fact, we treated multiples in our pilot plant unit with no apparent loss of insect killing power. The most likely process for citrus would be similar to the hot dog process that is currently under commercial development. In the commercial process, multiple unsealed packages will be treated on a conveyor. The line speed is expected to be 17–18 packages per minute. Each package of hot dogs weighs 454 g. The total treatment time for the packages of hot dogs is <2 s. Another rate comparison is for chicken carcasses to which this research was originally directed. Chicken line speed is currently ≈4,000 carcasses per hour. The optimum VSV processing conditions for chicken require <1 s.

Acknowledgment

We thank Janet Hare, Thomas Estrada, and Michael Clark for assistance in preparing scale infested lemons for shipment from California to Delaware. We are grateful to Isis Johnson for assistance in examining and counting the numerous scales that had to be evaluated. In addition, we are indebted to E. Richard Radewonuk who ran the VSV process. Maintenance of armored scale colonies was funded by the Citrus Research Board. Project 5500-157 ("Infrastructure for Armored Scale Research").

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